Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants

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ABSTRACT

Little is known about the physiological and biochemical responses of plants exposed to surface modified nanomaterials. In this study, tomato (*Solanum lycopersicum* L.) plants were cultivated for 210 days in potting soil amended with uncoated and citric acid coated cerium oxide nanoparticles (nCeO₂, CA+ nCeO₂) bulk cerium oxide (bCeO₂), and cerium acetate (CeAc). Millipore water (MPW), and citric acid (CA) were used as controls. Physiological and biochemical parameters were measured. At 500 mg/kg, both the uncoated and CA+ nCeO₂ increased shoot length by ~9 and ~13%, respectively, while bCeO₂ and CeAc decreased shoot length by ~48 and ~26%, respectively, compared with MPW ($p \leq 0.05$). Total chlorophyll, chlorophyll *a*, and chlorophyll *b* were significantly increased by CA+ nCeO₂ at 250 mg/kg, but reduced by bCeO₂ at 62.5 mg/kg, compared with MPW. At 250 and 500 mg/kg, nCeO₂ increased Ce in roots by 10 and 7 times, compared to CA+ nCeO₂, but none of the treatments affected the Ce concentration in above ground tissues. Neither nCeO₂ nor CA + nCeO₂ affected the homeostasis of nutrient elements in roots, stems, and leaves or catalase and ascorbate peroxidase in leaves. CeAc at 62.5 and 125 mg/kg increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg/kg, increased Ca in stems (84% and 86%, respectively). On the other hand, bCeO₂ at 62.5 increased Zn (152%) but reduced P (80%) in stems. Only nCeO₂ at 62.5 mg/kg produced higher total number of tomatoes, compared with control and the rest of the treatments. The surface coating reduced Ce uptake by roots but did not affect its translocation to the aboveground organs. In addition, there was no clear effect of surface coating on fruit production. To our knowledge, this is the first study comparing the effects of coated and uncoated nCeO₂ on tomato plants.

Keywords: Cerium oxide nanoparticles; surface coating; tomato plant life cycle; fruit production

1. Introduction

Cerium oxide nanoparticles (NPs) or nanoceria (nCeO_2) are amongst the top 10 nanomaterials produced worldwide (Keller and Lazareva, 2014). Similar to the bulk cerium, these nanoparticles (NPs) are mainly used in the automotive industry as catalysts or in electronics and optics. Keller and Lazareva (2014) estimated that in 2010, the global production nCeO_2 reached 10,000 tons of which 100 ended in air, 300 in water and 1,400 in soil. Engineered nanomaterials (ENMs) including nCeO_2 , have several applications; however, the uncoated forms tend to aggregate and overgrowth, which limit their performance. To improve their stability, ENMs are surface capped with several materials (Niu and Li, 2014). Citric acid (CA) is a common coating agent due to its stability and availability (Masui et al., 2002; Chanteau et al., 2009; Liu et al., 2012). However, coating molecules change the surface chemistry and interaction of ENMs with the environment (Chanteau et al., 2009).

Previous studies have shown that nCeO_2 have the potential to alter the physiology and biochemistry of plants. However, there is a lack of uniformity in the reported results and none of the parameters seem to be affected in the same manner when there are variations in species, growth media, and treatment concentration. Lopez-Moreno et al. (2010a) exposed nCeO_2 to several seeds in liquid medium and found that at 2000 mg/L, nCeO_2 reduced the germination of tomato (*Solanum lycopersicum*), corn (*Zea mays*), and cucumber (*Cucumis sativus*). Lopez-Moreno et al. (2010a) also reported an increase in cucumber and corn root seedling elongation but a reduction in alfalfa and tomato root length. On the other hand, Ma et al. (2010) reported that at 2000 mg/L, nCeO_2 reduced the root elongation in lettuce but not in tomato, radish (*Raphanus sativus*), wheat (*Triticum aestivum*), cabbage (*Brassica oleracea*), cucumber, and rape (*Brassica napus* L.).

A complete assessment of the effects of nCeO₂ on plants is difficult due to the lack of studies covering the entire life cycle. A review of current literature reported that by 2014, only 30 studies covered the effects of ENMs over the full life cycle of plants (Gardea-Torresdey et al. 2014). Of those, only five were about nCeO₂. Wang et al. (2012) exposed tomato in potting soil to consecutive applications of nCeO₂ suspension at 10 mg/L. These researchers reported no effects on plant growth and production; however, high Ce content was found in the fruit. Morales et al. (2013) reported that at 250 mg/kg, nCeO₂ decreased biomass and caused conformational changes in the macromolecular composition of cilantro. Rico et al. (2013a, 2014) reported changes in essential elements and other nutritional components in rice (*Oryza sativa*) and wheat (*Triticum aestivum*) grains. Zhao et al. (2014) reported 31.3% reduction in cucumber fruit production under exposure to 800 mg nCeO₂/kg; Corral-Diaz et al. (2014) also exposed nCeO₂ (500 mg/kg) to radish and reported no effects in production but changes on the antioxidant power of radish tubers. Rico et al. (2015) reported that nCeO₂ increased plant biomass in *Hordeum vulgare*, but inhibition of grain formation in plants exposed to 500 mg/kg.

Several reports have also shown that nCeO₂ affect the activity of stress enzymes. Zhao et al. (2012b) reported that catalase (CAT) and ascorbate peroxidase (APOX) activities increased up to day 15 in shoots of corn seedlings exposed to nCeO₂ at 800 mg/kg soil. Rico et al. (2013b) found a decrease in CAT activity, yet an increase in APOX activity in rice roots exposed to 500 mg nCeO₂/kg soil. Majumdar et al. (2014) reported a decrease in APOX in kidney bean leaves of plants exposed for 15 days to 250 and 500 mg nCeO₂/kg .

A few studies have shown the effects of surface coating on the interaction of ENMs with plants. Zhao et al. (2012a) reported that the uptake of Ce by corn plants exposed to alginate coated nCeO₂ was driven by the soil organic matter. In a more recent study, Trujillo-Reyes et al.

(2013) found that the Ce uptake by radish was significantly lower in plants exposed to citric acid coated nCeO₂, compared to uncoated NPs. Continuous increments in the applications of coated CeO₂ NPs increase the chances for their build up in the environment, which could results in unpredicted effects on crop plants. In addition, Hernandez-Viezcas et al. (2013) have shown that nCeO₂ taken up by crop plants are stored without changes in plant organs. Tomatoes are berry-type fruits widely consumed in raw form. Thus, they could become a carrier of nCeO₂ into the food chain.

In this research, effects of Ce compounds/NPs on the growth, fruit production, uptake of Ce and essential elements, as well as chlorophyll content and the activity of CAT and APOX enzymes were measured in fully developed tomato plants.

2. Materials and methods

2.1 Preparation of nanoparticle suspensions and other treatments

Uncoated CeO₂ NPs (nCeO₂) (10 nm, Meliorum Technologies, Rochester, NY) were obtained from the University of California Center for Environmental Implications of Nanotechnology (UC CEIN). According to a previous characterization (Keller et al., 2010), these nCeO₂ have primary size of 8 ± 1 nm, particle size of 231 ± 16 nm in DI water, surface area of $93.8 \text{ m}^2 \text{ g}^{-1}$, and 95.14% purity. Citric acid coated CeO₂ NPs (CA+nCeO₂, 1:2 ratio) were prepared and characterized according to Trujillo-Reyes et al. (2013). Enough particles were suspended in an 8:2 v/v water: ethanol solution to reach a 0.001 M concentration. Nanoparticles were sonicated (Crest Ultrasonics, Trenton, NJ) in a water bath for 60 minutes at 20°C with a sonication intensity of 180 watts. Another 8:2 v/v water: ethanol solution was prepared with enough citric acid to reach a concentration of 0.002 M. The reaction was adjusted to pH 7-8 with a 3M NaOH solution. Both solutions were mixed and maintained in reflux for 3 hours. At last,

ethanol evaporated, and the CNPs were oven dried at 65°C for 24 hours. Suspensions/solutions of NPs or compounds including nCeO₂, CA+nCeO₂, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA) were prepared with MPW in order to add to each pot 0, 62.5, 125, 250 and 500 mg/kg of the respective compound. Each pot was irrigated with 450 ml of the corresponding suspension/solution. These concentrations were selected after Rico et al. (2013b). The calculations were done according to the amount of potting soil used per pot (~450 g). Suspensions were stirred and sonicated for 30 min to avoid aggregation before homogeneous mixing with the soil.

2.2 Seed Germination and plant growth

Seeds of tomato (*Solanum lycopersicum*), Roma variety, were purchased from Del Norte Seed & Feed (Vinton, TX). Seeds were placed in a beaker with MPW and stirred for 3 hours until hydrating. One thousand, six hundred and eighty grams of Miracle-Gro® organic potting mix were separated, put in a glass container, and mixed with the Ce treatments. A brief description of the Miracle-Gro® is shown in Table S9 of the supplementary data. Four hundred and twenty grams of the Ce amended soil and control soil were placed in each pot, creating four replicates per treatment, except the MPW control that had 16 replicates, four for each Ce compound/NP. The soil was left for 24 hours for conditioning before planting.

For germination, seeds of approximately the same size and aspect were selected. Five seeds per replicate/treatment were used. The seeds were placed about 2.5 cm deep in the soil and watered with 100 mL of MPW every day. Pots were placed in a greenhouse with 14-h photoperiod, 25/20°C day/night temperature, 70% relative humidity under light intensity of 340 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The seed germination began on the third day and the stem length was recorded at 15, 30, 60, 120, and 210 days after germination (DAG). At 60 days, four seedlings were removed

and only the biggest plant in each pot was cultivated to full maturity. The number of fruits per plant and the percent of mature fruits from day 139 to 210 after germination were also recorded.

2.3 Quantification of Ce, nutrients, and Al in dry plant tissues

At harvest (210 days), roots, stems and leaves were washed with a 5% CaCl_2 solution and rinsed three times with MPW. Samples were dried for 72 hours in an oven at 60° C, and grinded with mortar and pestle until powdered. Samples of 0.2 g of tissues were microwave acid-digested by adding 1 mL of plasma pure HNO_3 and 4 mL of 30% hydrogen peroxide in a microwave oven (MarsX, CEM Corporation Mathews, NC). The digests were diluted to 50 mL with MPW. Micro and macro nutrients, aluminum, and cerium quantification in the acidic solutions was performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 4300 DV, Shelton, CT). Blanks, spikes and standard reference materials NIST 1547 and peach leaves, (Gaithersburg, MD) were used to validate the digestion and analytical methods for Ce, Al, B, Ca, Cu, Fe, K, Mg, Mn, P and Zn. For QA/QC, ICP readings of a blank and a standard were done every 15 samples.

2.4 Catalase (CAT) and ascorbate peroxidase (APOX) assays

A previous study showed differential effects of the nCeO_2 concentrations on different stress enzymes in rice plants (Rico et al. 2013b). In this study we determined the activity of catalase (EC 1.11.1.6) and ascorbate peroxidase (EC 1.11.1.11) in leaves of 210-day old tomato plants grown in potting soil amended with the different Ce-based compounds/NPs. Fresh leaves were washed with a 5% CaCl_2 solution and MPW three times to remove external contaminants. For each sample, 0.2 g of fresh leaves were grinded in a mortar and pestle and extracted with 1800 μL of a phosphate buffer solution (25 mM KH_2PO_4 at pH 7.4). Extracts were centrifuged for 10 min at -4°C and 9,600 rpm (Eppendorf AG bench centrifuge 5417 R, Hamburg,

Germany). The supernatants were then transferred to 2 mL Eppendorf tubes to continue with the assay. Catalase (CAT) activity was done according to Gallego et al. (1996). A 950 μL aliquot of 10 mM H_2O_2 was placed in a quartz cuvette, and an aliquot of 50 μL of the sample was added to obtain a final volume of 1 mL. The mixture was shaken three times by hand, and the absorbance at 240 nm was recorded for three min in a Perkin Elmer Lambda 14 UV/Vis Spectrometer (single-beam mode, Perkin-Elmer, Uberlinger, Germany). The amount of protein for CAT/APOX was determined by the fresh weight of the tissue employed.

The APOX activity was evaluated according to Murguia et al. (2004). Extract of fresh tomato leaves were prepared as described previously Rico et al. (2013b). The supernatant was separated by centrifugation. An aliquot of 4 μL of 25 mM ascorbate, 10 μL of 17 mM H_2O_2 , 886 μL of 0.1 M KH_2PO_4 buffered at pH 7.4 and 100 μL of fresh leaf extract were placed in a quartz cuvette and mixed three times. The absorbance was recorded at 265 nm for 2 min in a Perkin Elmer Lambda 14 UV/Vis Spectrometer. The absorbance was recorded as described above.

2.5 Chlorophyll content

Total chlorophyll, chlorophyll *a* and *b* (chlo-*a* and chlo-*b*) contents were determined as per Porra et al. (2002). Fresh tomato leaf tissue was cryogenized with liquid nitrogen, and later employed for extractions. A sample of 0.5 g of leaf tissue was grinded with 80% acetone for chlorophyll extraction. The extracts were kept in a freezer at -80°C until the assay was performed. The absorbance at 663 and 646 nm was measured using a Perkin Elmer Lambda 14 UV/Vis Spectrometer.

2.6 Statistical Analysis

Four replicates of each treatment concentration were allocated in a completely random design in the greenhouse facility. Data was analyzed using one-way ANOVA (PASW Statistics

18 software) and the Tukey's HSD test at $p \leq 0.05$ was used to determine statistical differences between treatment means. Data presented are mean $\pm$ standard errors (SE) of four replicates.

3. Results and Discussions

3.1 Cerium concentration in tissues

Figure 1 shows the cerium concentration in roots, stems and leaves of 210-day old tomato plants grown in soil amended with uncoated and coated nCeO₂, bCeO₂, and cerium acetate at 0 to 500 mg/kg. In this study, no Ce was detected in plants exposed to citric acid. As seen in Figure 1, some treatments affected the Ce accumulation in vegetative organs, compared to their respective MPW control. In roots, there was a concentration-dependent increase of Ce that was statistically higher in plants exposed to nCeO₂ at 125, 250 and 500 mg/kg ($\sim 41 \pm 8.1$, 130 ± 18.0 and 197 ± 20 mg/kg d wt, respectively), compared to control. Moreover, root Ce at 250 and 500 mg/kg of nCeO₂ was statistically higher, compared to 62.5 and 125 mg/kg. At all concentrations CA + nCeO₂, bCeO₂ and CeAc significantly increased Ce concentration in roots, compared with their respective controls (Figure 1A). The data suggests that the concentration of Ce in roots was not associated with the solubility of the compounds. The solubility of nCeO₂ is 1.28 g/L (Dahle and Arai, 2015) and the solubility of CeAc is 3.5 g/L (<http://www.gelest.com/goods/pdf/metalOrganicCatalog/58.pdf>). The difference could be due to a high absorption of uncoated NPs plus particles adsorbed to the root surface that were not removed by the washing process. The surface coating significantly reduced the Ce uptake by roots (Fig 1A). The Ce concentration in roots of plants exposed to coated NPs was seven times lower than in plants exposed to uncoated NPs (Table S5). This could be a result of the different interactions of coated and uncoated NPs with the root surface, due to the ζ potential of the particles. Uncoated nCeO₂ had a ζ potential of 20.1 ± 1.2 mV and the ζ potential of coated

nCeO₂ was -57 ± 0.6 mV (Trujillo-Reyes et al. 2014). Thus, the negative surface charge of the root plasma membrane (Wang et al. 2014) repelled the negatively charged coated NPs. Trujillo-Reyes et al. (2014) found similar results in radish exposed to citric acid coated nCeO₂. Similar results were also reported by Zhao et al. (2012a) in corn roots exposed to alginate coated nCeO₂.

Previous studies have shown that nCeO₂ tend to remain in roots (Wang et al. 2012; Zhao et al. 2012a; Schawbe et al. 2013; Chichiricco and Pomma, 2015). Zhao et al. (2012a) reported that the translocation of Ce in corn plants exposed to alginate coated nCeO₂ was driven by the soil organic matter. These authors found that shoots of plants grown in low organic matter soil amended with 200 and 400 mg/kg of alginate coated nCeO₂ “had 104 and 106%, respectively, more Ce compared with plants grown in organic soil.” Trujillo-Reyes et al. (2014) exposed uncoated and citric acid coated nCeO₂ to radish seedlings in hydroponics. Authors did not report translocation as they measured the whole seedling; however, they found 94% less Ce in plants exposed to coated NPs. In our study, none of the treatments showed high Ce translocation to the above ground tissues. In stems, Ce concentrations were, in general, < 0.8 mg/kg d wt, while in leaves were ≤ 2 mg/kg d wt (Table S5) In stems, only the nCeO₂ at 125 and 500 mg/kg ($\sim 0.67 \pm 0.03$ and 0.61 ± 0.07 mg/kg d wt, respectively) showed a statistical difference with respect to the MPW control ($\sim 0.36 \pm 0.07$ mg/kg d wt). Uncoated nCeO₂ also increased at 62.5 mg/kg in tomato leaves when compared to the control (2.1 ± 0.7 and 0.9 ± 0.2 mg/kg, respectively) (Figures 1B-C). In addition, none of the treatments showed Ce accumulation in fruit. This result differs from the result reported by Wang et al. (2012) who reported “substantially higher Ce concentrations” in fruit of plants that were fed with 130 mg of CeO₂/kg of dry potting mix during the entire life cycle. Perhaps the difference was due to the exposure methodology. In our study, the whole amount of NPs was applied to the soil 24 hours before seeding, while Wang et al. fed

the plants twice a week until harvesting with a NP suspension at 10 mg/L. In addition, the Miracle-Gro potting mix has a high content of organic matter (50-60 percent forest products, as shown in Table S9), which has been shown to bind NPs (Grillo et al. 2015)

3.2 Chlorophyll content in leaves

Figure 2 shows total chlorophyll, chlo-*a*, and chlo-*b* in leaves of tomato plants cultivated in soil amended with nCeO₂, CA + nCeO₂, bCeO₂, CeAc and citric acid (Table S6). As seen in Figure 2 (A-C) only the bCeO₂ at 62.5, 250 and 500 mg/kg affected the chlorophyll production. At 62.5 mg/kg the bCeO₂ treatment significantly reduced total chlorophyll and chlo-*a*, compared with control, 250 and 500 mg/kg. This suggests less production of ATP that can affect the general performance of the plants (Rabinowitch and Govindjee, 1965). However, at 250 and 500 mg/kg from bCeO₂ increased total chlorophyll and chlo-*a* with respect to the MPW control. Our results concur with previous reported results with nCeO₂. Zhao et al. (2014, 2015) reported that nCeO₂ did not alter leaf net photosynthetic rate, gas exchange, stomata conductance, transpiration rate and total chlorophyll content in cucumber and corn. These results contrast with previous studies which note that nanoparticles procure a negative effect on the chlorophyll content (Perreault et al., 2010; Rico et al., 2013b; Mohammed et al., 2011; Mazumdar et al., 2014). This corroborate that the response to NPs varies with several factors, including the growth medium, the environment, and plant species. In the present study, the surface modification of nCeO₂ did not change the impact on chlorophyll production.

3.3 Catalase and Ascorbate peroxidase activities

The defense mechanism of plants is sometimes activated by environmental, biological, or chemical stress. Catalase and ascorbate peroxidase are enzymes that deal with stress by fighting

the reactive oxygen species (ROS) generated by the plants in the form of H_2O_2 (Panda, 2005).

Figure 3 (A-B) shows the activity of CAT and APOX in leaves of 210-day old tomato plants. As seen in this figure, none of the concentrations of $nCeO_2$ significantly affected CAT activity, compared with control; while coated NPs at 500 mg/kg, significantly increased CAT, compared with control. It is possible that at concentrations < 500 mg/kg, the plant could cope with the stress imposed by the CA + $nCeO_2$. On the other hand, the activity of APOX showed the same reduction pattern under exposure to both uncoated and coated NPs, except at 62.5 mg/kg, where coated NPs did not affect APOX activity. Differences in CAT activity on plants exposed to uncoated and coated NPs suggest that the coating reduced the CAT mimetic activity $nCeO_2$ (Pirmohamed et al., 2010). However, it seems that the coating does not reduce the peroxidase-like activity of $nCeO_2$, as APOX activity in tomato showed, in general, the same pattern.

Catalase activity was significantly reduced by $bCeO_2$ at 125 mg/kg, but surprisingly, increased at 250 and 500 mg/kg, although the difference at 500 g/kg did not reach statistical significance, compared with control. Similarly, at 125 mg/kg, CeAc significantly increased CAT activity, compared with control (Figure 3A). The effects of bCe and CeAc were different on APOX activity. While practically all $bCeO_2$ concentrations significantly reduced the activity, only the highest concentrations of CeAc (250 and 500 mg/kg) significantly reduced APOX activity, compared with control. In CeAc, cerium is in the trivalent state (Ce(III)), which easily binds to phosphates and hydroxides and has lower catalase mimetic activity (Pirmohamed et al., 2010), while in $bCeO_2$ it exist as both Ce(III)/Ce(IV) that has shown to have superoxide dismutase and peroxidase scavenging activity (Rico et al., 2015). This can explain the effects of both compounds on CAT and peroxidase activities.

None of the citric acid concentrations modified CAT activity, and only at 250 mg/kg, there was a significant reduction in APOX activity, compared with control (Figure 3B). However, the difference was not clear as the average obtained at 250 mg/kg, overlap with the averages observed at other concentrations, and these overlapped with control. Previous reports have shown different effects of nCeO₂ on plants. Further comparisons between the treatments can be observed in the Table S7. Morales et al. (2013) did not report changes on CAT activity in cilantro exposed to 0 to 500 mg/kg in similar soil to the one used in this study. Zhao et al. (2012b) cultivated corn in soil amended with 400 and 800 mg nCeO₂/kg. These researchers reported an increase in H₂O₂, concomitant with increases in CAT and APOX, but only in 10-day old plants, suggesting that corn rapidly generated an adaptive response to the stress imposed by the NPs.

3.4 Nutrient element accumulation

Previous studies have shown that ENMs alter the uptake and translocation of nutrient elements. In the present study, all macro and microelements were analyzed in root, stem, leaf, and fruit (Table 1 and Supplementary data Tables S1-S3). However, only Al (a non-essential element), B, Ca, Fe, P and Zn showed statistically significant differences, compared with control (Table 1). As seen in this table, CeAc at 62.5 and 125 mg/kg increased B (81%) and Fe (174%) in roots, while at 250 and 500 mg/kg, increased Ca in stems (84% and 86%, respectively). On the other hand, bCeO₂ at 62.5 increased Zn (152%) but reduced P (80%) in stems. Coated nCeO₂ increased Al in roots (175%) and leaves (180%). CeAc has a K_{sp} of 0.35 g/100 g H₂O; this means that the acetate ion may function as a chelating agent for cations, increasing their absorption. It is not clear how the coated NPs increased Al absorption; however, in a previous study Trujillo-Reyes et al. (2013) found that citric acid coated nCeO₂ increased Al

uptake by 93% in radish. Perhaps the negative surface charge of the coated NPs bound Al, facilitating its uptake. More studies are needed in order to elucidate these results.

3.5 Stem growth

Table 3 shows the stem length of tomato plants at 15, 60, and 210 days after germination. As one can observe in this table, both the uncoated and coated NPs affected the growth at 60 days after germination. At this stage, uncoated NPs at 62.5 and 125 and coated at 62.5 mg/kg reduced stem growth, compared with control. Mixed results were observed at 210 days; however, at 500 mg/kg uncoated and coated NPs increased stem length by 9 and 13%, respectively, compared with MPW control. The current data is not enough to explain the effects on stem elongation as, practically, the NPs did not affect chlorophyll contents and nutrient uptake. A previous study showed that nCeO₂ reduced radish root biomass and stem length, while citric acid coated nCeO₂ increased root biomass (Trujillo-Reyes et al. 2013). The results observed with coated NPs in tomato do not seem to be driven by the external citric acid that could be released by the coated NPs. Mudunkotuwa and Grassian (2010) have shown that at the pH used in this study, citric acid is fully deprotonated and tightly bound to NPs. Then, it could be due to surface modifications that interfered with other functions of the plants not analyzed in this study.

Citric acid showed no significant effect at 15 days but mixed results at 60 days, while concentrations of 250 and 500 mg/kg produced significant reduction in stem elongation at 120 days, but the plants recovered at 210 days (Table S8). Citric acid is normally synthesized by tomato plants and can protect the plants by chelating excess of elements (Table S4), or it can help the plant to uptake some elements found at low concentrations in the soil solution (Senden et al., 1995). In this study, citric acid did not increase elements in tissues, neither affect chlorophyll nor CAT and APOX. Thus, this should be the reason why at the end of the cycle,

plants were not affected. Bulk cerium also showed mixed results at 30 days. However, at 120 and 210 days, all concentrations showed a consistent and significant stem reduction, compared with control and the other treatments. At 210 days, bCeO₂ and CeAc decreased shoot length by ~48 and ~26%, respectively, compared with MPW ($p \leq 0.05$). Our data concurs with the data reported by Majumdar et al. (2014) who found that in red kidney bean there was a decrease in stem biomass in plants exposed to bCeO₂, compared to nCeO₂. Majumdar et al. (2014) also reported a correlation among the stress and the reduction in biomass. In tomato, bCeO₂ at 250 and 500 mg/kg significantly increased CAT but reduced APOX, which could be the reason for the stem growth reduction. Cerium acetate also reduced stem length in adult plants exposed to 250 and 500 mg/kg. Cerium acetate (Ce³⁺), has shown to have superoxide scavenging activity but not catalase activity (Pirmohamed et al., 2010). Due to that, in general, CeAc did not affect CAT activity, but reduced APOX activity at 250 and 500 mg/kg. This reduction on APOX activity prevents the reduction of H₂O₂ generated by SOD into H₂O (Rico et al., 2015). Excess of H₂O₂, one of the reactive oxygen species, is translated in toxicity, which in turn reduced the growth of stems.

3.6 Fruit production

Table 2 shows the absolute number of ripe fruits collected from the tomato plants exposed to the different treatments. Although the greenhouse had good conditions for the plant to grow, the light intensity (340 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was not high enough to support good fruit production as tomatoes grow better under full light exposure. Thus, this table shows the total number of fruits, but the data was not representative for a statistical analysis. The data gathered showed that most of control plants got mature fruits in normal period (<http://tchester.org/analysis/tomatoes/>). The percent of ripe tomatoes in MPW control treatments

at 151 days varied from 57% (controls for coated NPs) to 100% (controls for CeAc treatments). Interestingly, all uncoated and coated NP treatments, except 500 mg/kg, had ripe tomatoes at 151 days. However, plants exposed bCeO₂ at 62.5 did not produced ripe tomatoes at 151 days, plants exposed to 125 mg/kg did not produce any tomatoes, while plants exposed to 250 and 500 mg/kg had 50% and 40% ripe tomatoes, respectively, at 151 days. It is worth noting that plants exposed to coated NPs, except at 125 mg/kg, had blossom end rot. This is a serious tomato disorder associated with Ca deficiency that can affect more than 50% of production (<http://ohioline.osu.edu/hyg-fact/3000/pdf/3117.pdf>). However, none of the NPs interfered with Ca accumulation in fruit (data not shown); in addition, there was a regular water supply, and the pH was around 6.5 (Table S9). This suggests that other factors were involved in the induction of the blossom end rot, which deserves additional investigation.

4. Conclusions

The data of this study suggests that tomato stem elongation, in fully developed plants, was enhanced at the highest concentration of both coated and uncoated nanoparticle treatments, but was reduced by bCeO₂ and CeAc compounds at 210 days after germination. The citric acid coating did not have effect on chlorophyll *a*, *b* and total chlorophyll contents of tomato.

However, the surface coating had effect on the biochemical response of the plant as coated NPs increased CAT activity at 500 mg/kg. On the other hand, at 125 mg/kg, bCeO₂ decreased CAT activity by 83.90%. Both coated and uncoated NPs showed similar reducing effects on APOX, except at 62.5 mg/kg, where coated NPs did not affect APOX. In addition, all bCeO₂ concentrations and CeAC at 250 and 500 mg/kg reduced APOX activity. ICP-OES results demonstrated that the coating reduced Ce uptake by roots but did not have effect on its translocation to the aboveground plant parts. Neither uncoated nor coated nCeO₂ affected the

homeostasis of nutrient elements in roots, stems, and leaves, and there was no clear effect of surface coating on the fruit production of tomato plants. To our knowledge, this is the first life cycle study comparing the effects of coated and uncoated nCeO₂ on tomato plants.

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Figure legends

Figure 1. Ce concentration in roots (A), stems (B), and leaves (C) of tomato plants grown to full maturity (210 days) in soil amended with 0 to 500 mg/kg of uncoated (nCeO₂), citric acid coated (CA + nCeO₂) NPs, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates $\pm$ SE. Different letters indicate statistically significant differences between concentrations of the same treatment at ($p \leq 0.05$); $n = 4$. Citric acid was not included in the figure as it does not contain cerium.

Figure 2. (A) Chlorophyll *a*, (B) chlorophyll *b*, and (C) total chlorophyll contents in leaves of 210 day-old tomato plants grown in soil amended with uncoated (nCeO₂), citric acid coated (CA + nCeO₂) NPs, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA). Data are means of three replicates $\pm$ SE. Different letters indicate statistically significant differences between concentrations from the same treatment at ($p \leq 0.05$); $n = 3$.

Figure 3. Antioxidant activity of (A) catalase and (B) ascorbate peroxidase in fresh leaves of 210 day-old tomato plants grown in soil amended with uncoated (nCeO₂), citric acid coated (CA + nCeO₂) NPs, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates $\pm$ SE. Different letters indicate statistically significant differences between concentrations from the same treatment at ($p \leq 0.05$); $n = 4$.

Table 1. Micro- and macro- elements altered in 210 day-old tomato plants exposed to uncoated (nCeO₂) and citric acid coated (CA + nCeO₂) nanoparticles, cerium acetate (CeAc), and bulk CeO₂ (bCeO₂). Data are average $\pm$ SE of four replicates, except control (Millipore water) that had 16 replicates. Comparisons were made with respect to the controls and symbols + and – stand for percent of increase and decrease in nutrient concentration.

Organ	Element	Treatment (mg/kg soil)	Concentration (mg/kg d wt tissue)	%
Roots	Al	Control	1732.3 $\pm$ 171.6	100
		Coated NP 62.5	4760.0 $\pm$ 1122.2	174.8 +
		CeAc 62.5	5130.4 $\pm$ 820.0	196.2 +
	Fe	Control	1268.1 $\pm$ 104.2	100
		CeAc 62.5	3470.1 $\pm$ 488.9	173.6 +
	B	Control	30.5 $\pm$ 2.7	100
Stems	Ca	CeAc 125	55.0 $\pm$ 3.2	80.5 +
		Control	10473.5 $\pm$ 540.3	100
		CeAc 250	19295.1 $\pm$ 2587.2	84.2 +
	P	CeAc 500	19501.0 $\pm$ 255.82	86.2 +
		Control	39993.1 $\pm$ 255.8	100
	Zn	bCeO ₂ 125	7853.1 $\pm$ 594.8	80.4 -
		Control	71.9 $\pm$ 7.7	100
Leaves	Al	bCeO ₂ 62.5	181.6 $\pm$ 17.5	152.5 +
		Control	189.2 $\pm$ 13.4	100
		Coated NP 250	530.1 $\pm$ 131.8	180.2 +

Table 2. Total production, percentage of mature fruits at 151 and 210 days, and fruits with blossom end rot at 210 days in tomato plants grown in soil amended with 0 (control) to 500 mg/kg of uncoated (nCeO₂), citric acid coated (CA + nCeO₂) NPs, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA). The control treatment was watered with Millipore water. DAG stands for days after germination.*Total number of tomatoes for 4 replicates.

Treatment	mg/kg	No. tomatoes*	Mature (139-151 DAG)	Never ripened (210 DAG)	Blossom end rot
			%	%	%
nCeO ₂	0	6	83.3	16.7	0
	62.5	18	38.9	27.8	0
	125	6	50	16.7	0
	250	9	66.7	11.1	0
	500	5	0	40	0
CA + nCeO ₂	0	7	57.14	14.3	14.3
	62.5	8	37.5	37.5	12.5
	125	5	40	0	0
	250	8	25	0	25
	500	6	0	0	16.7
bCeO ₂	0	4	75	25	0
	62.5	3	0	100	0
	125	0	0	0	0
	250	4	50	0	0
	500	5	40	20	0
CeAc	0	5	100	0	0
	62.5	4	25	0	0
	125	5	0	0	0
	250	3	0	33.3	0
	500	6	33.3	16.7	16.7
CA	0	8	62.5	12.5	0
	62.5	6	16.7	0	0
	125	1	0	100	100
	250	12	50	25	0
	500	1	0	0	0

Table 3. Shoot length of 15, 60, and 210 day-old tomato plants grown in soil amended with uncoated (nCeO₂), citric acid coated (CA + nCeO₂) NPs, bulk CeO₂ (bCeO₂), cerium acetate (CeAc), and citric acid (CA). Data are means of four replicates $\pm$ SE. Different letters indicate statistically significant differences between concentrations at ($p \leq 0.05$); $n = 4$.

		Control	62.5	125	250	500
nCeO ₂	15	17.33 $\pm$ 0.30	15.94 $\pm$ 0.07	14.73 $\pm$ 1.00	16.33 $\pm$ 0.19	16.96 $\pm$ 0.74
	60	49.65 $\pm$ 0.21a	40.49 $\pm$ 0.93 b	39.55 $\pm$ 0.84 b	44.11 $\pm$ 1.94 ab	46.10 $\pm$ 0.90 ab
	210	146.31 $\pm$ 0.27 b	135.99 $\pm$ 0.03b	112.21 $\pm$ 0.15c	142.21 $\pm$ 1.30 b	162.20 $\pm$ 1.80 a
CA + nCeO ₂	15	14.38 $\pm$ 0.22	14.57 $\pm$ 0.16	14.66 $\pm$ 0.46	16.13 $\pm$ 0.48	14.76 $\pm$ 0.04
	60	47.22 $\pm$ 1.02 a	41.66 $\pm$ 1.51 b	43.25 $\pm$ 1.05 ab	44.53 $\pm$ 0.67 ab	43.26 $\pm$ 0.43 ab
	210	145.64 $\pm$ 1.12 c	136.06 $\pm$ 2.41 d	130.09 $\pm$ 1.07 d	158.61 $\pm$ 1.85 b	168.49 $\pm$ 1.73 a
bCeO ₂	15	13.26 $\pm$ 0.20	13.54 $\pm$ 0.87	14.48 $\pm$ 1.15	15.91 $\pm$ 0.26	16.46 $\pm$ 0.79
	60	49.76 $\pm$ 0.21 a	44.54 $\pm$ 0.75 b	39.68 $\pm$ 0.72 b	31.62 $\pm$ 0.48 c	30.57 $\pm$ 0.36 c
	210	149.19 $\pm$ 1.47 a	89.48 $\pm$ 0.55 b	83.11 $\pm$ 0.02 bc	81.55 $\pm$ 0.20 bc	78.13 $\pm$ 0.05 c
CeAc	15	15.03 $\pm$ 0.44	15.19 $\pm$ 0.18	15.11 $\pm$ 0.04	16.37 $\pm$ 0.22	15.04 $\pm$ 0.85
	60	48.68 $\pm$ 0.47	46.91 $\pm$ 0.71	45.05 $\pm$ 1.49	46.33 $\pm$ 1.12	47.75 $\pm$ 0.68
	210	144.38 $\pm$ 0.25 a	140.31 $\pm$ 0.02 ab	135.10 $\pm$ 1.16 bc	127.00 $\pm$ 0.49 c	106.31 $\pm$ 1.53 d
CA	15	15.27 $\pm$ 0.29	15.43 $\pm$ 0.09	16.28 $\pm$ 0.43	16.39 $\pm$ 0.81	16.03 $\pm$ 0.37
	60	49.33 $\pm$ 1.05 bc	40.22 $\pm$ 0.82 c	45.28 $\pm$ 0.73 ab	54.55 $\pm$ 0.30 a	52.88 $\pm$ 0.32 a
	210	144.38 $\pm$ 0.21	148.65 $\pm$ 1.47	144.74 $\pm$ 1.65	156.04 $\pm$ 0.77	156.81 $\pm$ 1.23

Figure 1.

Figure 2.

A CAT

□ Control ■ 62.5 ■ 125 ■ 250 ■ 500

B APOX **Figure 3.**